SimPlate®®
Total *Enterobacteriaceae* Color Indicator

Introduction

SimPlate® for Total *Enterobacteriaceae* Color Indicator (Entero-CI) method is used for the detection and quantification of the total *Enterobacteriaceae* populations. It is based on Binary Detection Technology (BDT) which equates the presence of total *Enterobacteriaceae* to the presence of a color change in the medium. The medium/sample mixture is dispensed into a SimPlate® device and incubated for 24–28 h. The total *Enterobacteriaceae* count is determined by counting the wells with changed color and referring to the SimPlate® Conversion Table. Colored wells indicate the presence of total *Enterobacteriaceae*. The SimPlate® device is packaged separately.

Single Test Medium

Kit Components

100 individually-packaged dehydrated Entero-CI medium containers.

A. Sample Preparation

a. Weigh 50 g of sample into 450 mL of sterile diluent (Butterfield's phosphate buffer, maximum recovery diluent, or peptone salt solution). This is a 10-fold dilution. Masticate or blend to homogenize.

b. If an alternate sample size is specified in your testing procedure or standard, prepare a 10% weight to volume suspension.

c. If necessary, prepare 10-fold serial dilutions appropriate for the anticipated population of the sample.

B. Test Procedure

For 1.0 mL sample size

a. Resuspend powdered medium with 9.0 mL of sterile deionized water. Add 1.0 mL of sample and mix well. DO NOT count this reconstitution as a dilution.

For 0.1 mL sample size

b. Resuspend powdered medium with 9.9 mL of sterile deionized water. Add 0.1 mL of sample and mix well. This is an additional 10-fold dilution.

NOTE: The final volume of sample/medium mixture in the container should be 10 ±0.2 mL.

Multiple Test Medium

Kit Components

50 multi-test dehydrated Entero-CI medium containers. Each container is sufficient for 10 tests.

A. Sample Preparation

a. Weigh 50 g of sample into 450 mL of sterile diluent (Butterfield's phosphate buffer, maximum recovery diluent, or peptone salt solution). This is a 10-fold dilution. Masticate or blend to homogenize.

b. If an alternate sample size is specified in your testing procedure or standard, prepare a 10% weight to volume suspension.

c. If necessary, prepare 10-fold serial dilutions appropriate for the anticipated population of the sample.

B. Test Procedure

a. Empty contents of one container into 100 mL of sterile deionized water. Shake to completely dissolve.

b. Remove the lid from the SimPlate® device. If prepared sample size is 1.0 mL, pipette it onto the center of the device (Figure 2). Overlay the sample with 9.0 mL of medium. DO NOT count this medium addition as a dilution.

c. For 0.1 mL of prepared sample, overlay it with 9.9 mL of medium: this is an additional 10-fold dilution.

c. Remove the lid from the SimPlate® device and pour the sample/medium mixture onto the center of the plate (Figure 1). Immediately replace the lid.

Note: The final volume of sample/medium mixture on the plate should be 10 ±0.2 mL. Immediately replace the lid.

d. Gently swirl to distribute the sample/medium mixture into all the wells (Figure 3). The plate may be held with both hands and tilted slightly to help distribute the liquid into the wells.

e. If necessary, tap the SimPlate® device GENTLY on a hard surface to remove any air bubbles which may have become trapped in the wells (Figure 4). Do not be concerned if partially filled wells are present. Wells containing partial volume of liquid will turn positive in the presence of viable bacteria.

f. Pour off excess medium by holding the lid against the plate on either side of the sponge cavity. Tip the plate toward you to allow liquid to drain into the sponge (Figure 5). Observe the background color of the wells. Background is defined as the color of the sample/medium mixture inside the wells.

g. DO NOT invert the SimPlate® device. Incubate in the dark for 24 to 28 h at 37° ±1 °C.

Figure 1

For single test, pour sample/ medium mixture onto the center of the plate.

Figure 2

For multiple tests, pipette sample onto center of plate. Add rehydrated medium to make a final volume of 10 ± 0.2 mL.

Figure 3

Cover plate, gently swirl to distribute the sample into all of the wells.

Figure 4

Tap plate GENTLY on a hard surface to remove air bubbles.

Figure 5

Holding the cover, tip the plate toward you to allow liquid to drain.

C. Reading and Interpretation of Results

a. After incubation, observe color change of the liquid in the wells. Disregard particulate matter if present. Count the number of wells showing a color change from the background color of the medium/sample mixture. Wells with changed color correspond to the presence of *Enterobacteriaceae.*

b. To determine the population, perform the following calculations:

1. Count the number of positive wells on the plate.
2. Use the SimPlate® Conversion Table to determine the total number of microorganisms per plate.

   To calculate the number of **microorganisms per g (mL)** multiply the count in **C(b)**(2) by the appropriate dilution factor (see section **A** and **B**).

D. Product and Storage Information

a. Store dehydrated medium away from direct light between 2–30 °C.

b. DO NOT use expired medium.

c. Store containers of reconstituted medium in the dark between 15 and 25 ºC and use within 12 h.

d. Handle and dispose of incubated medium in a decontamination container and sterilize according to Good Laboratory Practices.

Manufacturing Entity

BioControl Systems, Inc, 12822 SE 32nd St, Bellevue, WA 98005, USA.

BioControl Systems, Inc is an affiliate of Merck KGaA, Darmstadt, Germany.

MilliporeSigma
400 Summit Drive
Burlington, MA 01803

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